DEVELOPMENT OF AUTOMATIC COUNTING AND IDENTIFICATION TRAP FOR MOSQUITO MONITORING

Dong Gun Kim[1], Hyun Jung Kim[2], Sumi Na[2], Jae Seung Yoo[3], Yeon Jae Bae[4] and Hoonbok Yi[2]

[1]Smith College of Liberal Arts, Sahmyook University; [2]Department of Bio and Environmental Technology, Seoul Women's University, Seoul; [3]E-TND, Environmental Technology and Development, Kwangju City, Kyunggi-do; [4]Division of Environmental Science and Ecological Engineering, Korea University, Seoul, Republic of Korea

Abstract. To efficiently collect mosquito monitoring data, an automatic device was developed with intention to attract and catch female mosquitoes, count the number of trapped mosquitoes, and identify mosquitoes through image analysis. In addition, the device was able to send collection and identification data to an offsite server via a long-term evolution router module (WCDMA; Wideband Code Division Multiple Access, CDMA; Wideband Code Division Multiple Access). Compared to manual inspection, the device selectively attracted mosquitoes at a rate of 80 ± 24% and, based on image analysis, classified mosquitoes with 90% accuracy. The device was designed as a combined information and communication technology research tool to facilitate long-term mosquito monitoring and could serve as a basis for the establishment of a model surveillance system for vector-borne infectious diseases.

Keywords: counting, identification, monitoring, mosquito, surveillance

INTRODUCTION

Global warming is becoming increasingly serious due to an increase in greenhouse gases, such as carbon dioxide (CO_2), methane, nitrous oxide, and ozone (IPCC, 2014). These climatic changes affect the composition of the atmosphere and climate variability. A rapid increase in mean temperature in the Korean Peninsula due to global warming may cause unexpected changes and disturb endemic biological systems (Parmesan and Yohe, 2003; Root *et al*, 2003). Furthermore, according to the Korea Institute for Health and Social Affairs (KIHASA, 2012), increases in temperature and precipitation lead to changes in ecosystems and increased frequency of vector-borne diseases in the country. Due to global warming, the worldwide mosquito population has increased at a rate faster than that of the development of control measures (Khasnis and Nettleman, 2005).

With continued global warming, Korea will likely experience an influx

Correspondence: Hoonbok Yi, Department of Bio and Environmental Technology, Seoul Women's University, Seoul 01797, Republic of Korea.
Tel: +82 2970 5668; Fax: +82 2970 5974; Email: yih@swu.ac.kr

of invasive pathogens, including *Aedes albopictus* Skuse, 1894, a major vector of dengue fever and zika virus, which could be found throughout South Korea; *Culex pipiens* Linnaeus, 1758, a West Nile virus vector; chigger mite, a vector of scrub typhus; and *Ixodes scapularis* Say, 1821, a tick-borne vector of encephalitis and Lyme disease (Githeko *et al*, 2000; Tatem *et al*, 2006; Hong and Seo, 2011; Ogden *et al*, 2014; Kraemer *et al*, 2015). In South Korea, the current system for monitoring mosquitoes consists of two research divisions and three organizational divisions (community health centers, the Research Institute of Public Health and Environment, and the Centers for Disease Control and Prevention) that separately deal with related infectious diseases (*Anopheles hyrcanus* and *Culex pipiens* groups). This system is hindered in conducting effective

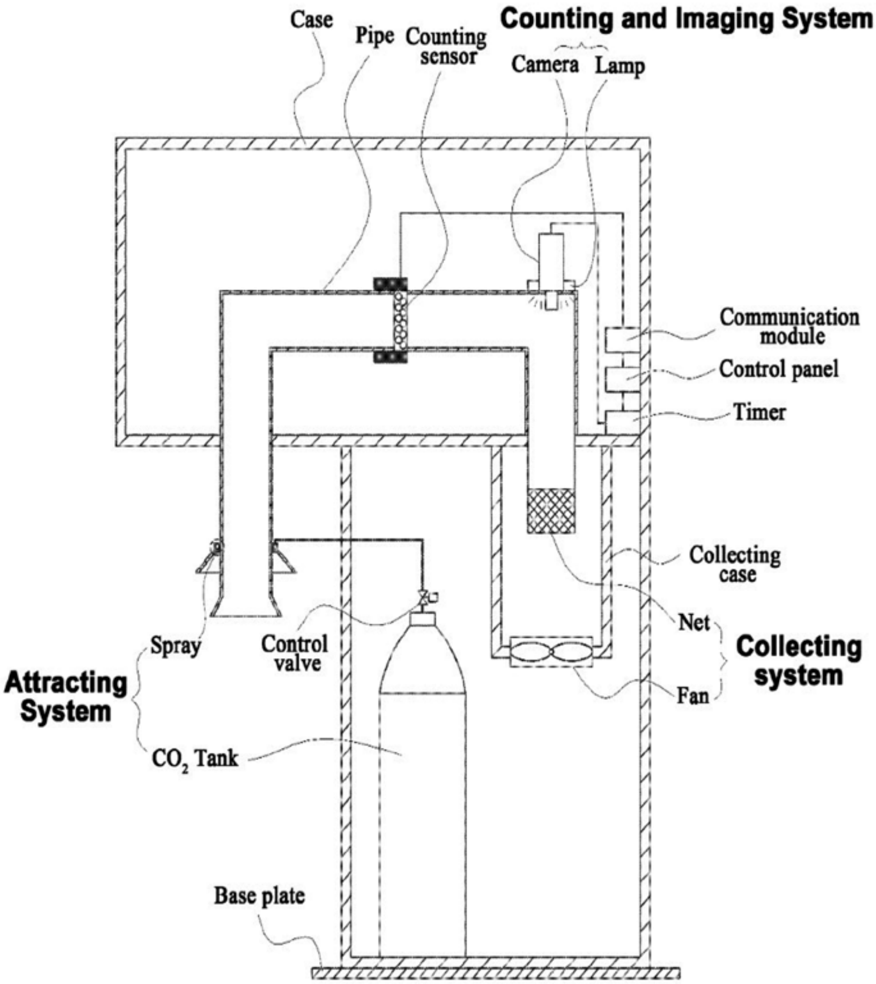

Fig 1-Diagram of the automatic counting and identification device showing the four components: attracting system, counting system, imaging system, and collecting system.

and objective analyses of monitoring results because of issues that include an appreciable lack of human resources, delays in obtaining monitoring results, and the lack of scientific validation of new collection tools.

With the aim of developing a mechanism to enable the rapid implementation of appropriate intervention and control response measures, we designed a mosquito surveillance trap that attracts, traps, counts, and identifies female mosquitoes, and then transmits the data via a long-term evolution router module (WCDMA; Wideband Code Division Multiple Access, CDMA; Wideband Code Division Multiple Access) to an offsite server.

MATERIALS AND METHODS

Construction of the device

The device consisted of four systems: an attracting system, a counting system, an imaging system, and a collection system (Fig 1). The attraction component uses CO_2 gas to attract host-seeking mosquitoes and then aspirates the adult mosquitoes into a collecting bag. This component consists of a circular inlet surrounded by a nozzle containing tubing that releases specific attractants. The counting system contains a sensor, which counts mosquitoes as they are aspirated into the tube. The imaging system consists of a digital camera and a light emitting diode lamp. The mosquitoes are photographed as they pass through the chamber and into the collection net. The net is attached to a square plastic frame and retains mosquitoes and other insects entering the trap. A bottom-up airflow configuration was used to minimize the aspirates from other flying insects that could be attracted to the trap (Fig 2). The digital camera was mounted vertically 20 cm above the net. Photographs were

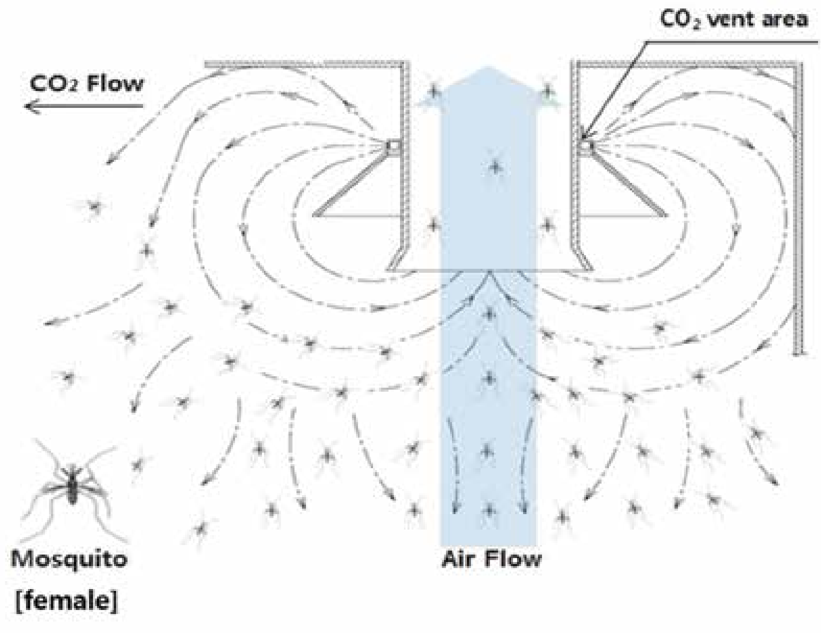

Fig 2-Diagram showing CO_2 gas flow from the CO_2 vent and suction of mosquitoes.

acquired at 10-minutes intervals with illumination provided by lamps installed around the net. Following photography, the net was rotated 180° and specimens were blown down into the collecting net, thereby preparing the opposite side of the net for newly aspirated insects and the next image capture. The sequence and timing for photography was controlled using a fully automated central control circuit. The collection system comprised a 1 mm mesh bag that trapped mosquitoes for subsequent morphological identification and counting in a laboratory, as well as for vector count confirmation and detection of other pathogens.

Digital images were sent to a server and mosquito species were identified using an algorithm with the following workflow: 1) images were cleared of noise by applying a Gaussian filter and image reduction; 2) a binary search was performed to detect the mosquitoes and if an adjacent region existed in the binary image, it was classified as the same individual; 3) size of the specimen was used as a reference to exclude non-mosquito individuals; 4) using individuals assigned to the mosquito-candidate group, a normal distribution graph was plotted based on the center of gravity, and those matching the mosquito shape were selected and subjected to the final identification process; and 5) regions corresponding to the selected individuals were extracted from the original image, cleared of noise, and subjected to red, green and blue (RGB) histogram analysis

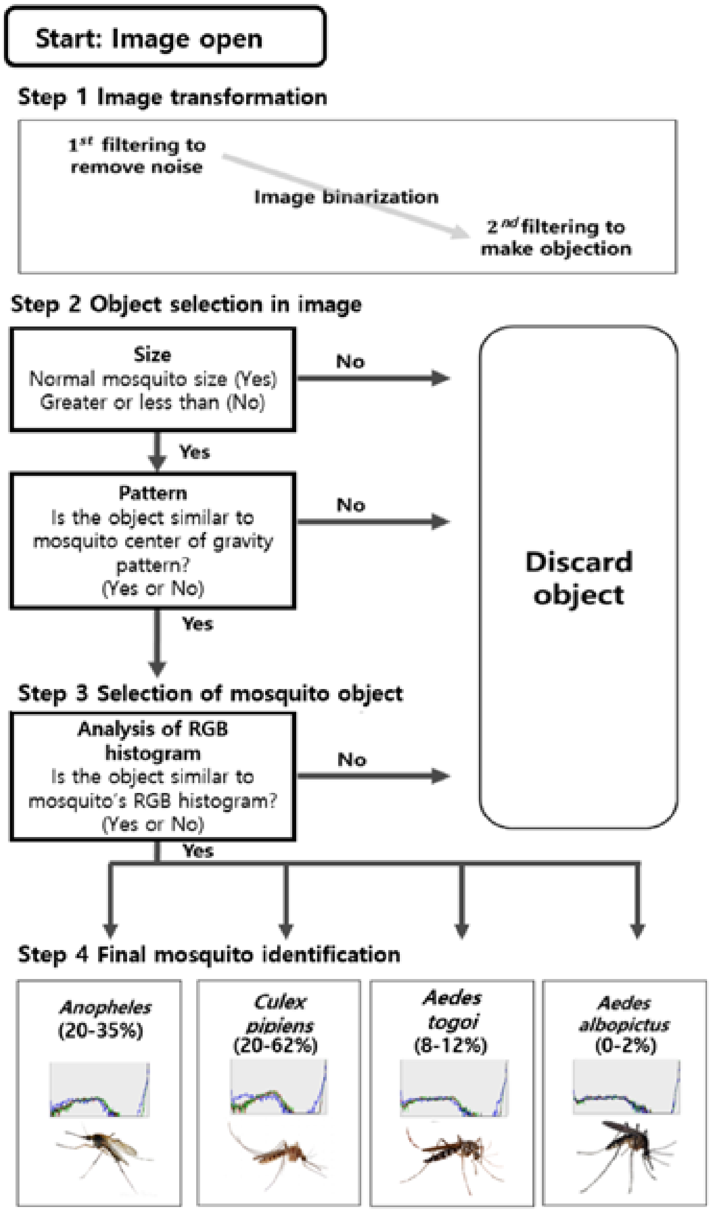

Fig 3-Workflow of the automatic image analysis algorithms used for mosquito identification.

(Fig 3). Individuals not matching the green and red patterns common to mosquitoes were excluded and those remaining after the exclusion process were considered mosquitoes and species identification was done through a refined RGB histogram analysis.

RESULTS

To identify effective attractants, an analysis was conducted to evaluate components from a human blood-meal source. Attractants that included hexanoic acid, lactic acid, and octenol were excluded because they might attract other biting fly species, such as *Culicoides* spp, deer flies, and horse flies (Wang *et al*, 2006). A CO_2 emission rate of 300 ml/minute was selected as the primary attractant to maximize collection counts (McPhatter and Gerry, 2017). Ultraviolet light (356 nm) was excluded as it attracted a large number of non-target specimens (Table 1). A fan was used to create suction of 2 meters/second to prevent damage to the captured mosquitoes. Other collection methods, such as glue-paper or electric shock, were excluded because of the difficulty in isolating individuals and the damage caused to specimens (Table 2).

The four major mosquito species targeted in the identification process - *Aedes albopictus*, *Aedes togoi*, *Culex pipiens*, and *Anopheles* spp - were collected in urban areas of South Korea. All species were clearly distinguishable by the blue and gray arrangement in the RGB patterns. Based on the arrangement demonstrated by *Ae. albopictus* (0-2%), *Ae. togoi* (8-12%), *Cx. pipiens* (20-62%), and *An.* spp (20-35%) (Fig 3), *Ae. albopictus* and *Ae. togoi* were clearly distinguishable, while *An.* spp and *Ae. togoi*, which partially overlapped, were distinguished by comparing the patterns across the RGB histograms. Accuracy of the image analysis was

Table 1
Analysis of factors determining selection of attractants for collecting female mosquitoes.

Cause of mosquito bloodsucking	Mosquito attractant	Characteristic	Decision
Host respiration	Carbon dioxide, octenol	Pulmonary breathing emits carbon dioxide Ruminant animals emit octenol	Selected
Host skin respiration	Lactic acid, hexanoic acid, moisture	Emitted from sweat Microbial decomposition product	Pending
Host metabolism	Host body temperature, hormones	Leads to direction of landing	Testing
Host characteristics and environmental factors	Color, skin condition, wind (aerodynamics)	Detection factor, helpful for approaching host	Selected
Insect photo-periodism	Ultraviolet light (365 nm)	No selectivity	Pending

Table 2
Comparison of mosquito-collecting methods.

Collecting method	Medium	Characteristic		Decision
			Bottom →otop	Selected
Air suction	Air	Air-flow	Top →obottom	Pending
			Side →ibottom	Pending
Glue-paper	Glue-paper adhesion	Random adhesion of insects		Not selected (no individual isolation)
Electric shock	Electric charge (> 1,000 V)	Electric shock by high voltage		Not selected (sample destroyed)

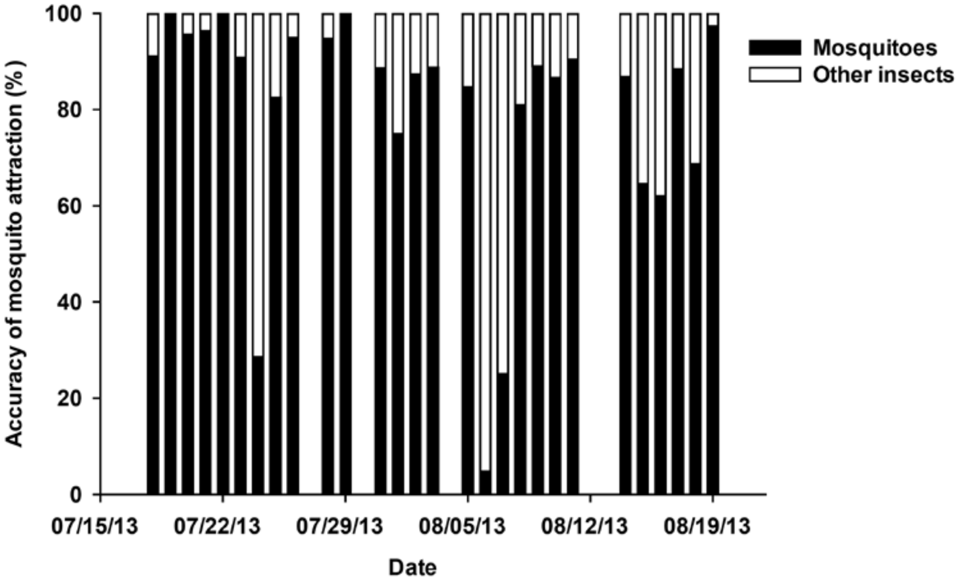

Fig 4-Accuracy of mosquito automated counting and identification device.

Note: Percent accuracy of identification was compared to manual identification.

confirmed by comparing aspects of visual assessment results, such as wing scales, leg patterns, and proboscis shapes, with those of the digitally acquired values. To test the practical efficiency of the device, several collection trials were established in urban areas during peak mosquito season (July and August) in 2013. The device selectively attracted mosquitoes at a rate of 80 ± 24% (Fig 4) with the digital identification accuracy of 91 ± 16% using imaging analysis (Fig 5).

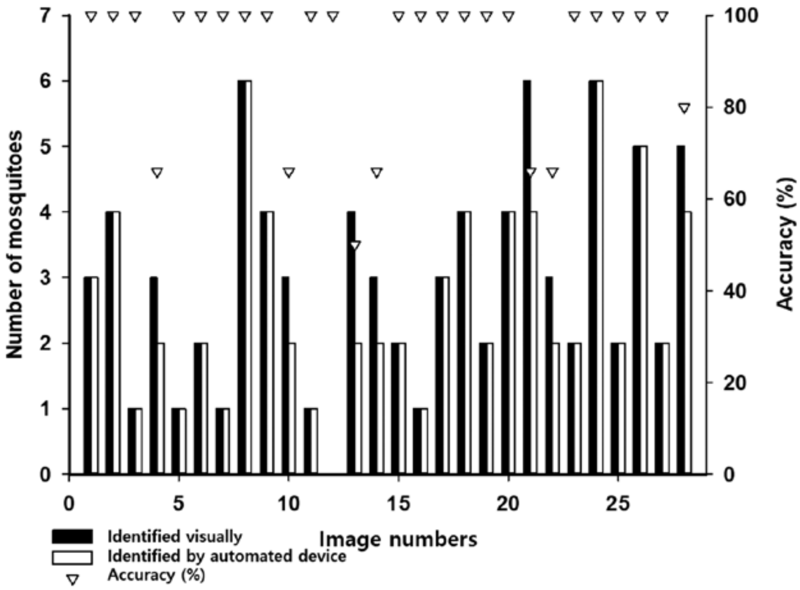

Fig 5-Comparison of mosquito counting between the automated counting system and visual method.

DISCUSSION

The device was developed to meet the needs for an objective and highly efficient monitoring tool for disease vectors in high-risk areas and to provide real-time surveillance results. Several studies are presently attempting to integrate biological information (*eg* pests) with information and communication technology. Devices with validated applicability are being developed for this purpose (Alphey, 2014; Abad-Franch, 2016; WHO, 2016).

The device developed in this study meets leading global standards, by integrating biological technology (scientifically based pest collection techniques) and information acquisition and transmission technologies. By enabling the long-term monitoring of the changing behaviors of vector mosquitoes and data-based objective observations, the device could serve as the basis for the establishment of a leading system for the surveillance of vector-borne infectious diseases to develop and implement infectious disease mitigation strategies.

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